RESEARCH CONFERENCES

ESF-EMBO Symposium

Antiviral RNAI:From molecular biology towards applications

Pultusk, Poland
11-15 June 2012

Chair: Dr. Juan Antonio Garcia, Centro Nacional de Biotecnologia-CSIC, Campus de la Universidad Autonoma, ES
Co-Chair: Professor Ben Berkhout, Academic Medical Center, University of Amsterdam, NL
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Highlights & Scientific Report

Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

Virus infections form a major threat for humans. This includes viruses that are endemic in the human population and causes a large number of casualties each year, like influenza virus, hepatitis B and C viruses (HBV, HCV) or human immunodeficiency virus (HIV), but also continuous emergence of new viruses and new viral strains. Virus affecting animals and plants cause very important economic losses, something with a high social impact. In addition, many animal viruses constitute reservoirs from which human-infecting strains with high pathogenic and epidemic potential can emerge.

We now know that RNA interference (RNAi) is an essential mechanism to control gene expression and cell differentiation in many organisms ranging from yeast to human. RNAi plays a major role in antiviral defenses of different eukaryotic organisms both with general and host-specific mechanistic features. Thus, affecting the machinery of RNAi and its virus-related targets could have a profound impact on approaches to combat viral infections. Moreover, RNAi-related biotechnological tools have been shown to be able to silence host and viral factors required for viral infection.

The objective of this conference was to bring together virologists from all fields from plants to mammals, and covering the virus-RNAi interplay from basic mechanisms to clinical applications. This objective was reached as there have been contributions on RNA and DNA viruses infecting plants, worms, insects and mammals, including humans, from quite different virus families (*Caulimoviridae*, *Tombusviridae*, *Potyviridae*, *Bromoviridae, Rhabdoviridae*, *Dicistroviridae*, *Nodaviridae*, *Birnaviridae*, *Picornaviridae*, *Flaviviridae*, *Hepadnaviridae*, *Togaviridae*, *Retroviridae*, *Paramyxoviridae*, *Herpesviridae*, etc).

Important results were reported on: i) basic elements of the RNAi-related defense-counterdefense mechanisms, such as novel small and long-noncoding RNAs, novel silencing suppressors, host targets of viral and host miRNAs, ii) technical tools to assess antiviral RNAi, such as deep sequencing of small or long RNAs, or the design of valuable viral RNAi vectors, iii) virus evolution to escape from antiviral RNAi, iv) interaction of RNAi with other antiviral defenses in natural infections, mainly the interferon system, and in biotechnological antiviral approaches, such as the synergistic therapeutic effect of a combination of RNAi and the new U1i mechanism, v) clinical trials to assess the efficiency of antiviral RNAi against important human infections, like those caused by HIV, hepatitis C virus (HCV), and respiratory syncytial virus (RSV).

ESF-EMBO meetings around the same topic were organized by us in 2008 and 2010 and the participants sincerely hope that similar meetings could continue at regular intervals in the future.

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Scientific Report

Executive Summary

(2 pages max)

The meeting hosted 52 participants, including 23 invited speakers. All speakers were asked to stay around for some time after their lecture in order to foster further interactions with the participants, which worked out nicely. Most participants were from academia, but there was a keynote speaker from Santaris Pharma who reported interesting results on the application of antiviral RNAi research. Many new and exciting scientific discoveries were presented at the meeting. The invited speakers were allowed to speak for 25 minutes. We strictly kept to the time allotted to speakers to allow at least 5 minutes of discussion per lecture. In addition, the chairs selected 12 short talks (15 + 5 minutes) from the submitted abstracts. Many of the participants contributed to the discussions, and the chairs were specifically asked to first allow young participants (not sitting in the front rows) to ask their question. This worked out nicely and each talk in each session received numerous questions.

All 22 posters were presented in 3 evening sessions. Participants of whom the abstract was selected for a short oral presentation were specifically asked to also bring their poster to foster further discussions. These poster sessions were well attended and triggered many lively discussions, perhaps facilitated by the free beer in two of the sessions made possible through company sponsoring. A 3-person poster prize committee was formed on the spot with expertise from the different fields (from plant to human). Three poster prizes (250, 150 and 100 €), made possible by sponsoring of Oxford University Press (Nucleic Acids Research journal), were awarded on the third evening.

A number of young scientists were able to come to this meeting because of 16 fellowships that were made possible by the funding raised by the chairs. In addition to the regular ESF-EMBO funds, this included financial support from the Foundation for Polish Science, Society of Chinese Bioscientists in America, RNA Society, Oxford University Press and Elsevier. The chairs selected fellowship awardees based on: 1. A match of their research with the topic of the meeting, 2. Active participation in the meeting (poster presentation, of which some were selected for an oral presentation), 3. Facilitation of the attendance of young researchers, 4. Gender balance, and 5. When funds were limited we preferred to help European scientists.

The European COST action FA0608 is currently running on the topic of “Antiviral RNAi vaccination approaches in plants”, and two of the meeting chairs (García and Berkhout) also participate in this action. We intertwined a mini COST-meeting, open also to all the ESF-EMBO participants, on the afternoon of the second day of the conference. We organized the ESF-EMBO meeting such that the related plant lectures were presented in the morning and evening sessions surrounding this COST-afternoon, which allowed the COST-only participants to benefit from these lectures that are of direct relevance to them. The combined COST-ESF-EMBO action also allowed the chairs to invite more speakers because of the extra COST funds.

Scientific Content of the Conference

(1 page min.)

- *Summary of the conference sessions focusing on the scientific highlights*
- *Assessment of the results and their potential impact on future research or applications*

The 3-day meeting was organized globally in 5 sessions: I. RNAi and viral infections in mammals, II. RNAi and viral infections beyond mammals, III. Counter defence and counter-counter defence in viral infection, IV. Interplay between RNAi and other antiviral defence mechanisms, V. Towards therapeutics.

Many truly novel findings were reported, of which only a brief summary can be presented.

Mark Kay (Standford University, USA) reported on attempts to target the minus-strand RNA instead of the positive-strand RNA genome of hepatitits C virus (HCV). The idea is that the minus-strand is an essential replication intermediate and present at a low concentration, thus forming an attractive target for RNAi attack. However, evidence was presented that the minus-strand cannot be targeted. Explanations for this "bio-inaccessibility" include the following possibilities: the minus-strand RNA is inaccesible because it is covered by protein or always paired with the plus-strand, or the minus-strand RNA is located in a sub-cellular compartment that is not accessible for the RNAi machinery

A major challenge for RNAi applications *in vivo* is how to avoid off target effects of both the guide strand and passenger strand of the artificial siRNAs directed to the target gene. **Ying Poi Liu** (University of Amsterdam, The Netherlands) has discovered that depending on specific structural features, some short hairpin (sh) RNAs are not cleaved by Dicer into a 21-nt siRNA duplex, but rather processed into ~30-nt single stranded oligonucleotides - probably by the slicer activity of AGO2 - and subsequently loaded into active RISC complexes. These shRNAs, named AgoshRNAs, which are not producing passenger strands, are expected to cause reduced off target effects.

Marie-Anne Felix (CNRS-University of Paris-Diderot, France) presented pioneering research on the first identification of viruses in *Caenorhabditis elegans*. This nematode forms an ideal model system to study antiviral immunity and host-pathogen co-evolution because it would combine a genetically tractable small animal with a virus capable of naturally infecting the host organism. However, the use of *C. elegans* as a model to define host-viral interactions has been limited by the lack of viruses known to infect nematodes. From wild isolates of *C. elegans* and *C. briggsae* with unusual morphological phenotypes in intestinal cells, this group identified two novel RNA viruses distantly related to known nodaviruses, one infecting specifically *C. elegans* (Orsay virus), the other *C. briggsae* (Santeuil virus).

Tamas Dalmay (University of East Anglia, United Kingdom) focused his presentation on the extensive bias of deep sequencing of small RNAs. Using the standard adaptors to prepare siRNA libraries from randomised oligonucleotide substrates, the count distribution of sequence reads was significantly different from the expected Poisson distribution, and only 40% of the possible distinct sequences were captured. High definition (HD) adapters with four random terminal nucleotides drastically reduced the sequencing bias. These are important lessons as very many laboratories are currently performing RNA deep sequencing experiments.

Santiago Elena (Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, Spain) has implemented a method to identify virus sequence variants at frequencies as low as $2x10^{-6}$ and to track their variation in time by deep sequencing analysis. He used this method to study the emergence of virus mutants that escape from amiRNA-mediated resistance in plants. Maximal level of viral heterogeneity was reached in a single plant passage, and every site in the amiRNA target sequence presented variation in the viral population, even in the absence of selection pressure. These evolutionary insights will help to design improved and more durable antiviral strategies.

Puri Fortes (CIMA/UNAV, Spain) showed a synergistic antiviral effect against hepatitis B virus (HBV) of RNAi and interference with recombinant U1 small nuclear RNAs (U1i), both in *in vitro* culture and in a mouse model system. The synergistic effect was observed only when each interference mechanism was directed towards a different target.

Cristoph Coch (University Bonn, Germany) presented an antiviral approach against influenza viruses. While most of the data presented during the conference were based on the RNAi mechanism, Coch and colleagues also described double-stranded RNA molecules carrying a triphosphate at the 3' end to induce the innate immune system by triggering RIG-I. The RIG-I inducers showed a therapeutic benefit in a mouse model for influenza virus infections.

Ryszard Adamiak presented a new tool to predict three-dimensional RNA structures, which is essential to understand their biological function, e.g. in the RNAi pathway. A number of bioinformatic tools have been proposed to explore structural databases in order to analyze various aspects of RNA tertiary structures. RNA FRABASE 1.0 is the first web-accessible database with an engine for automatic search of 3D fragments within RNA structures. The second version (RNA FRABASE 2.0) provides a wide spectrum of novel functionalities. An intuitionally operated web server platform enables very fast user-tailored search of three-dimensional RNA fragments, their multi-parameter conformational analysis and visualization, which should facilitate a variety of advanced RNA studies.

Troels Koch (Santaris Pharma, Copenhagen, Denmark) described a strategy to inhibit hepatitis C virus (HCV) by oligonucleotides modified with Locked Nucleic Acids (LNAs). A single stranded DNA/LNA mixmer was directed against the liver specific microRNA-122, on which HCV relies for its replication. A first clinical study demonstrated the safety of this approach and promising results were reported with respect to HCV inhibition.

John DiVicenzo (University of Tennessee, USA) reported on the development of an RNAi-based approach against the respiratory syncytial virus (RSV), which is particularly dangerous for newborns. This virus is a promising target for RNAi strategies since it only infects the outer cell layer of the lungs and can thus be reached with siRNAs. Clinical trials with healthy adult volunteers that were purposely infected with RSV demonstrated a therapeutic benefit.

Answering to the invitation of the editor of EMBO reports, Ronald van Rij (Radboud University, The Netherlands), Tamas Dalmay (University of East Anglia, United Kingdom) and Dirk Grimm (University of Heildelberg, Germany) promised to write a meeting report.

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

During the general discussion of the last session, the overall feeling was that, although the number of participants of this year's meeting was significantly lower than that of two years ago in Sant Feliu, the meeting was very exciting and fruitful, with ample discussions across the different disciplines (from plants to humans). There was a strong feeling that it would be worthwhile to continue with this conference series on antiviral RNAi in the future.

With regard to future perspectives in the field, it was argued that we are now at a critical step of data accumulation (e.g. based on deep sequencing) and that a qualitative jump would be required to integrate this information in complete biological pictures. It was suggested that further data on the cell biology of RNAi, its molecular interaction with viral infections, and the use of systems biology approaches could contribute to facilitate this jump towards the next level.

Since the possibilities that ESF will support a fourth antiviral RNAi meeting in 2014 are low, we discussed alternative options: EMBO workshop, EPSO-supported conference, OECD workshop, direct sponsorship of a scientific society etc.

We also considered the possibility of organizing the conference with an industrial partner, which was considered suitable as long as the science would prevail in the meeting program over the more business-related aspects.

As all three chairs have now acted a meeting chair, they considered it wise not to organize a future antiviral RNAi conference, but they would be very happy to offer their support to a new organizing team.

Several issues worth considering for a future meeting were brought up in the general discussion:

- The multi-discipline approach (from plants to humans) was considered to be an important hallmark of our meetings. It should be conserved and even enhanced by including new organisms, e.g. fishes.

- It would be interesting to include piRNAs and germ-line effects due to endogenous retroviruses in the program.

- It was suggested to broaden the scope of the conference, covering not only RNA interference but also other antiviral defence mechanisms related to nucleic acids (e.g. innate immunity).

- In agreement with the future perspectives stated above, we also encouraged the inclusion in the program of cell biology and system biology approaches to study the virus-RNAi interplay.

- To attrack a bigger audience, it would be important to select a nice location for the meeting, with good travel connections.

- Is there a need for a foresight-type initiative?

We continue to recommend a foresight-type initiative in translational research to bring nucleic acid-mediated therapeutic interventions from the laboratory into the clinic.

Business Meeting Outcomes

- *Election of the Organising Committee of the next conference*
- *Identified Topics*
- *Next Steps*

In spite of the intense discussion on the wish to continue this meeting series of antiviral RNAi and the different sponsoring strategies that were raised, concrete decisions on time-place-organizers-sponsors were not taken.

The participants will explore different possibilities and will coordinate their efforts through the current chairs until a new organizing team is formed.

Atmosphere and Infrastructure

- *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

The atmosphere of the meeting was excellent. The infrastructure of the venue facilitated the scientific and social interactions between the participants. The only complaints referred to the rather bad travel connections to Pultusk and the unavailability of Internet connection in the rooms

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